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A Randomized, Open-label Study of the Efficacy and Safety of AZD4547 Monotherapy Versus Paclitaxel for the Treatment of Advanced Gastric Adenocarcinoma with *FGFR2* Polysomy or Gene Amplification

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Keywords:	AZD4547, Clinical Efficacy, Fibroblast Growth Factor Receptor, Gastric Cancer, Fluorescence in Situ Hybridization
Abstract:	**Background**: Approximately 5–10% of gastric cancers (GCs) have a fibroblast growth factor receptor-2 (*FGFR2*) gene amplification. AZD4547 is

a selective FGFR-1, 2, 3 tyrosine kinase inhibitor with potent preclinical activity in *FGFR2* amplified gastric adenocarcinoma SNU16 and SGC083 xenograft models. The randomized Phase II SHINE study (NCT01457846) investigated whether AZD4547 improves clinical outcome versus paclitaxel as second-line treatment in patients with advanced gastric adenocarcinoma displaying *FGFR2* polysomy or gene amplification detected by fluorescence *in situ* hybridization.

Patients and Methods: Patients were randomized 3:2 (*FGFR2* gene amplification) or 1:1 (*FGFR2* polysomy) to AZD4547 or paclitaxel. Patients received AZD4547 80 mg twice daily, orally, on a 2 weeks on/1 week off schedule of a 21-day cycle or intravenous paclitaxel 80 mg/m2 administered weekly on Days 1, 8, and 15 of a 28-day cycle. The primary end point was progression-free survival (PFS). Safety outcomes were assessed and an exploratory biomarker analysis was undertaken.

Results: Of 71 patients randomized (AZD4547 n = 41, paclitaxel n = 30), 67 received study treatment (AZD4547 n = 40, paclitaxel n = 27). Among all randomized patients, median PFS was 1.8 months with AZD4547 and 3.5 months with paclitaxel (one-sided p-value = 0.9581); median follow-up duration for PFS was 1.77 and 2.12 months, respectively. The incidence of adverse events was similar in both treatment arms. Exploratory biomarker analyses revealed marked intratumor heterogeneity of *FGFR2* amplification and poor concordance between amplification/polysomy and *FGFR2* mRNA expression.

Conclusions: AZD4547 did not significantly improve PFS versus paclitaxel in gastric cancer *FGFR2* amplification/polysomy patients. Considerable intratumor heterogeneity for *FGFR2* gene amplification and poor concordance between *FGFR2* amplification/polysomy and *FGFR2* expression indicates the need for alternative predictive biomarker testing. AZD4547 was generally well tolerated.

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Original Article

A randomized, open-label study of the efficacy and safety of AZD4547 monotherapy versus paclitaxel for the treatment of advanced gastric adenocarcinoma with *FGFR2* polysomy or gene amplification

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Running title: AZD4547 in gastric cancer with *FGFR2* polysomy/amplification (59/60 max characters)

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Key message *(400/400 max characters incl. spaces)*

The selective fibroblast growth factor receptor [FGFR]-1, 2, 3 tyrosine kinase inhibitor, AZD4547, failed to improve progression-free survival versus paclitaxel in gastric adenocarcinoma patients displaying *FGFR2* polysomy or gene amplification. Intratumor heterogeneity of *FGFR2* amplification and poor concordance with *FGFR2* expression highlight the need for alternative predictive biomarker testing.

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Abstract *[285/300 words]*

Background: Approximately 5–10% of gastric cancers (GCs) have a fibroblast growth factor receptor-2 (*FGFR2*) gene amplification. AZD4547 is a selective FGFR-1, 2, 3 tyrosine kinase inhibitor with potent preclinical activity in *FGFR2* amplified gastric adenocarcinoma SNU16 and SGC083 xenograft models. The randomized Phase II SHINE study (NCT01457846) investigated whether AZD4547 improves clinical outcome versus paclitaxel as second-line treatment in patients with advanced gastric adenocarcinoma displaying *FGFR2* polysomy or gene amplification detected by fluorescence *in situ* hybridization.

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Results: Of 71 patients randomized (AZD4547 n = 41, paclitaxel n = 30), 67 received study treatment (AZD4547 n = 40, paclitaxel n = 27). Among all randomized patients, median PFS was 1.8 months with AZD4547 and 3.5 months with paclitaxel (one-sided p-value = 0.9581); median follow-up duration for PFS was 1.77 and 2.12 months, respectively. The incidence of adverse events was similar in both treatment arms. Exploratory biomarker analyses revealed marked intratumor heterogeneity of *FGFR2* amplification and poor concordance between amplification/polysomy and *FGFR2* mRNA expression.

Conclusions: AZD4547 did not significantly improve PFS versus paclitaxel in gastric cancer *FGFR2* amplification/polysomy patients. Considerable intratumor heterogeneity for *FGFR2* gene amplification and poor concordance between *FGFR2* amplification/polysomy and *FGFR2* expression indicates the need for alternative predictive biomarker testing. AZD4547 was generally well tolerated.

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ClinicalTrials.gov identifier: NCT01457846

Key words: AZD4547, clinical efficacy, fibroblast growth factor receptor, gastric cancer, fluorescence *in situ* hybridization

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Introduction

Fibroblast growth factors (FGFs) and their receptors (FGFRs) are instrumental in a number of normal biologic processes, and their dysregulation by mechanisms including activating gene mutations, gene amplification and gene fusions, is believed to drive human cancers, including gastric cancer (GC) [1–3]. Approximately 5–10% of gastric tumors have an *FGFR2* amplification [4, 5], which appears to confer poor prognosis [5–7].

AZD4547 is a selective FGFR-1, 2, 3 tyrosine kinase inhibitor that has displayed potent activity in preclinical studies. Cell lines of gastric adenocarcinoma possessing *FGFR2* amplification were sensitive to AZD4547, resulting in reduced cell proliferation and cell death [8]. Additionally, AZD4547 induced rapid tumor regression in two *in vivo* models of *FGFR2*-amplified GC [8].

The primary hypothesis of the SHINE study was that AZD4547 has the potential to provide clinical benefit in patients with advanced gastric adenocarcinoma with tumors displaying *FGFR2* polysomy or gene amplification selected by centralized fluorescence *in situ* hybridization (FISH) testing. Exploratory biomarker analyses were performed to further assess *FGFR2* amplification heterogeneity within tumor sections and concordance with *FGFR2* expression.

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Materials and Methods

Study design and patient selection

SHINE was a multicenter, randomized, open-label study performed in 56 centers in Asia, North America, and Europe (ClinicalTrials.gov registration: NCT01457846; National Cancer Institute protocol ID: D2610C00004).

Patients were recruited with locally advanced or metastatic GC with radiologically-confirmed progression after one prior chemotherapy regimen. Tumors were required to display either *FGFR2* polysomy or amplification determined from archival tumor block or fresh tumor biopsy. Patients with prior exposure to AZD4547 or any other FGFR inhibitor were excluded. Patients in the *FGFR2* amplification cohort were randomized 3:2 to AZD4547 or paclitaxel. Patients in the *FGFR2* polysomy cohort were randomized 1:1 to AZD4547 or paclitaxel.

Tumor FGFR status was determined by centralized FISH screening using a non-commercial kit (DAKO). *FGFR2* amplification and polysomy were classified as follows:

- *FGFR2* amplification: *FGFR2*/Spectrum Green-labeled centromere of chromosome 10 (CEN10) ratio ≥2 or *FGFR2* gene clusters in ≥10% tumor cells
- High polysomy: *FGFR2*/CEN10 ratio <2 and ≥4 copies of *FGFR2* in ≥40% tumor cells
- Low polysomy: *FGFR2*/CEN10 ratio <2 and ≥4 copies of *FGFR2* in 10–39% tumor cells.

The amplified cohort was further stratified into 'low' (*FGFR2*/CEN10 ratio ≥2 and <5) or 'high' (*FGFR2*/CEN10 ratio ≥5) strata. Subsequent changes to the scoring system are detailed in the supplementary Material.

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All patients gave written informed consent. The study was approved by the Institutional Review Board/Independent Ethics Committee at each study center and conducted in accordance with the Declaration of Helsinki.

Treatment schedule

Patients received either AZD4547 80 mg twice daily (BID), orally, on a 2 weeks on/1 week off schedule of a 21-day cycle or paclitaxel 80 mg/m^2 as a 1-hour intravenous infusion weekly on Days 1, 8, and 15 of a 28-day cycle. The dosing strategy for AZD4547 was based on a phase I dose-escalation study [9, 10].

Assessments

Patients underwent Response Evaluation Criteria In Solid Tumors (RECIST) assessments (ver. 1.1) at baseline and every 8 weeks thereafter using computerized tomography or magnetic resonance imaging. All assessments were carried out at the local sites and were not confirmed centrally.

Pharmacokinetic (PK) assessments included changes in blood-borne biomarkers (phosphates, basic fibroblast growth factor [bFGF], and FGF23. Adverse events (AEs) and clinical laboratory values were monitored throughout the study.

End points

The primary end point was progression free survival (PFS). Secondary end points included overall survival (OS), objective response rate (ORR), change in tumor size at 8 weeks, and the percentage of patients without progressive disease at 8 weeks.

Interim analysis

Prompted by slow recruitment, AstraZeneca and the Safety Review Committee agreed that it would be appropriate to conduct an unscheduled interim analysis of efficacy (based on average change in tumor size) and tolerability data. The results did not show superiority of

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AZD4547 over paclitaxel in patients with advanced GC tumors with *FGFR2* amplification and a decision was made to cease enrollment and close the study.

Exploratory biomarker analysis

FGFR2 expression in ribonucleic acid (RNA) extracted from tumor samples was analyzed using the nCounter® platform (NanoString Technologies®, Inc., Seattle, WA, USA).

For heterogeneity analysis, FISH-stained sections were scanned into the MIRAX Panoramic 250 Flash II (3D Histech) scanner at 40× magnification in the x, y, and z planes and analyzed using custom HALO v1.9 software (Indica Labs). All cells within the tumor compartment were classified as amplified or non-amplified, based on target:control probe ratio thresholds (*FGFR2:*CEN10 probe signals where ratio <2.0 = non-amplified and ratio ≥2.0 = amplified) and a visual heterogeneity map generated.

Statistical analysis

PFS, OS and ORR in all randomized patients were analyzed using Cox proportional hazards models with covariates for *FGFR2* strata and treatment. PFS, OS and ORR within *FGFR2* strata were estimated from Cox proportional hazards models fitted in the overall population with covariates for *FGFR2* stratum, treatment, and the treatment by *FGFR2* stratum interaction. The effect of AZD4547 on change in tumor size in all randomized patients, and within each of the *FGFR2* strata, was estimated from an analysis of covariance (ANCOVA) model that included terms for baseline tumor size (log transformed), time from baseline scan to randomization, *FGFR2* stratum, treatment and the treatment by *FGFR2* interaction, where appropriate.

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Results

Participants

A total of 960 patients had to be pre-screened for *FGFR2* status to enable 71 patients to be randomized (AZD4547 *n* = 41 [57.7%]; paclitaxel *n* = 30 [42.3%]; full analysis set (FAS); Figure 1). FISH re-scoring to detect *FGFR2* amplification identified three patients in the FAS who no longer met polysomy or amplification criteria and were excluded from the efficacy analysis that included *FGFR2* stratum as a factor in the statistical model.

Treatment groups were generally well balanced with respect to demographic characteristics (supplementary Table S1).

Efficacy

PFS and disease outcome

Disease progression was reported in 36 of the 38 patients (94.7%) in the AZD4547 arm and 26 of the 30 patients (86.7%) in the paclitaxel arm.

In the FAS, median PFS was 1.8 months in the AZD4547 arm and 3.5 months in the paclitaxel arm, with a median duration of follow-up of 1.77 months and 2.12 months, respectively (see Table 1 for amplified and polysomy cohorts). The difference in PFS was not statistically significant in favor of AZD4547 at the one-sided 10% level (*p*-value from Cox proportional hazards model=0.9581). The observed hazard ratio (HR) was 1.57 (80% CI, 1.12–2.21) for AZD4547 compared with paclitaxel (Figure 2).

The observed HRs for the polysomy and amplified groups were 1.87 (80% CI, 1.17–3.06) and 1.30 (80% CI, 0.81–2.12), respectively. No statistically significant difference in PFS in favor of AZD4547 was observed for AZD4547 versus paclitaxel in either the polysomy or amplified groups (one-sided *p*-values of 0.9562 and 0.7590, respectively).

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Complete response was not reported in any patient (Table 2). In the overall population, the ORR was 2.6% in the AZD4547 arm and 23.3% in the paclitaxel arm (0% and 20.0%, respectively [amplified cohort] and 5.0% and 26.7%, respectively [polysomy cohort]). The difference in ORR was not statistically significant in favor of AZD4547 at the one-sided 10% level (odds ratio 0.09, 80% CI, 0.02–0.35, one-sided *p*-value=0.9970).

There were a total of 27 deaths (71.1%) in the AZD4547 arm and 18 deaths (60.0%) in the paclitaxel arm. In the FAS, median OS was 5.5 and 6.6 months for AZD4547 and paclitaxel arms, respectively, with a median duration of follow-up of 4.8 months and 5.1 months, and the difference in OS was not statistically significant (Figure 3; HR 1.31; 80% CI, 0.89–1.95, one-sided *p*-value=0.8156). In the amplified and polysomy cohorts, there was no difference between treatment groups in terms of median OS (Table 1: HR 1.26; 80% CI, 0.72–2.25, one-sided *p*-value=0.7006 for the amplified cohort, and HR 1.36; 80% CI, 0.80–2.38, one-sided *p*-value=0.7697 for the polysomy cohort).

Analysis of the percentage change in tumor size at Week 8 did not show any statistically significant difference in favor of the AZD4547 arm compared with the paclitaxel arm (difference 39.44; 80% CI, 25.18–55.33, one-sided *p*-value=0.9999). Similar results were observed in the amplified (difference 39.21; 80% CI, 19.43–62.26, one-sided *p*-value=0.9965) and polysomy (difference 39.68; 80% CI, 19.38–63.45, one-sided *p*-value=0.9961) cohorts.

Safety

For those patients who received treatment, the median total duration of treatment was 50.5 days in the AZD4547 arm and 57.0 days in the paclitaxel arm. AEs and serious AEs related to study treatment occurred at similar rates in both treatment arms (supplementary Table S2).

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Biomarker analysis

PK findings were consistent with previous studies of AZD4547 [9] (see Supplementary Materials; Figure S1).

FGFR2 expression was assessed by nanostring analysis of RNA from 73 archival tumor samples, comprised of 56 tumor samples from patients randomized to AZD4547 or paclitaxel (n = 35 and n = 21, respectively), and an additional 17 samples from pre-screened patients who were not randomized (*FGFR2* copy number normal [CNN]). Overall, the analysis set consisted of 24 amplified, 29 polysomy, and 20 CNN samples.

A range of overlapping *FGFR2* expression levels were observed between the amplified and non-amplified tumor samples (Figure 4A), with only 6/24 amplified tumors having elevated *FGFR2* expression and, of these, only 5 having expression levels overlapping with SNU16- and KATOIII *FGFR2*-amplified GC cell lines, which are highly sensitive to AZD4547 induced growth inhibition [11]. There was no evidence of elevated *FGFR2* expression outside the amplified cohort (Figure 4A).

FGFR2 amplification was assessed in sections from seven tumor samples from the high amplification (*FGFR2*:CEN10 ratio >5) AZD4547 arm, as this represented the patient group most likely to respond to treatment. As a benchmark, image analysis of a tumor section from the AZD4547-sensitive SNU16 tumor xenograft model revealed that 100% of tumor cells displayed *FGFR2* amplification with a mean *FGFR2*:CEN10 ratio of 38. In the seven patient tumor sections examined, the number of tumor cells ranged from approximately 1500 to >41000, and representative FISH-stained sections revealed marked sub-clonal heterogeneity, with between 8% and 70% of the tumor cells displaying *FGFR2* amplification (Figure 4B). However, there was no clear correlation between the extent of sub-clonal heterogeneity and tumor shrinkage in response to AZD4547 (Figure 4C).

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Exploratory survival analysis

Details of the exploratory survival analysis of non-randomized patients who underwent FISH pre-screening in the SHINE study are shown in Supplementary Materials (Figure S2).

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Discussion

The efficacy of paclitaxel monotherapy in the SHINE study was consistent with data from other studies in a second-line setting. Median PFS and OS in the paclitaxel arm was similar to outcomes reported previously [12–16]. The trend towards shorter PFS and OS observed in the *FGFR2* amplified group, is in agreement with earlier studies in patients with *FGFR2* amplification [5–7].

In the current study, AZD4547 was not superior to paclitaxel, in contrast to preclinical findings [8, 17]. The poor association between *FGFR*2 amplification and elevated *FGFR*2 expression observed in the SHINE study, together with marked sub-clonal heterogeneity of *FGFR*2 amplification in tumor sections, contrasts markedly with the high and homogenous amplification and high *FGFR*2 expression observed in the SNU16 model. Although no correlation was observed between the level of sub-clonal heterogeneity and tumor shrinkage, the failure to adequately enrich for clonally amplified tumors is likely to be a factor in the failure to translate the preclinical efficacy of AZD4547 to the clinic and this is supported by results from a translational clinical study in which patients with high and clonal *FGFR2* amplification responded to AZD4547 [18]. It is possible that a high threshold exists for clonality of *FGFR2* amplification to sensitize to AZD4547.

Heterogeneity of gene amplification does not necessarily result in lack of clinical efficacy as *HER2* amplification and expression is heterogeneous in GC [19], yet patients with *HER2* amplification benefit from treatment with trastuzumab [20]. Hence the impact of heterogeneity on the predictive nature of a gene amplification biomarker may be target dependent. A limitation of this study is that the archival diagnostic tissue samples screened by FISH and the *FGFR2* status may not reflect the status of metastatic tumor sites at study entry. Clearly tumors with *FGFR2* amplification leading to elevated *FGFR2* expression do exist, but this appears to be at a very low prevalence. Consequently, there is a need for alternative

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predictive biomarker testing to more effectively enrich for this population prior to assessment of FGFR therapies.

Elevated plasma phosphate is a pharmacodynamic marker of interrupting FGF23 signaling through FGFR inhibition in the kidney [21, 22] and has been observed for other FGFR inhibitors [23, 24]. The intermittent dosing schedule allowed for elevations in plasma concentrations of phosphate during the on-drug period to normalize during the off-drug period.

This study illustrates the considerable operational challenge associated with recruitment of low prevalence patient groups into clinical studies. Centralized FISH testing identified patients with *FGFR2* amplification at an actual prevalence of 9%. However, attrition between FISH pre-screening and randomization resulted in an operational prevalence of 1%. Follow-up of screened patients showed a trend for *FGFR2* amplification being associated with poor prognosis which may have contributed to the higher than expected attrition rate.

The AE profiles for AZD4547 and paclitaxel were consistent with their known pharmacologic effects. The AZD4547 80 mg BID 2 weeks on/1 week off schedule was well tolerated and no new safety signals were identified compared with previous studies [9, 11, 25].

Conclusion

Treatment with AZD4547 did not improve PFS compared with paclitaxel in the overall population or in patients with *FGFR2* amplification or polysomy according to FISH selection. The safety profile demonstrated that AZD4547 is generally well tolerated. Exploratory analysis revealed discordance between *FGFR2* expression and *FGFR2* amplification in gastric tumors selected using focal FISH testing, which to a large extent reflected considerable intratumor heterogeneity. Failure to enrich for a clonally amplified population may have contributed to the failure of the SHINE study to demonstrate superiority of AZD4547 compared with paclitaxel.

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Neil Smith, Paul Frewer, Jayantha Ratnayake, Paul Stockman, Elaine Kilgour and Dónal Landers are employees of AstraZeneca. Elaine Kilgour and Paul Frewer hold AstraZeneca shares. Paul Stockman holds AstraZeneca shares.

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gastric (GC) and gastroesophageal (GOJ) cancer. ASCO Annual Meeting, Chicago, IL, 30 May–3 June, 2014 (abstract).

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Figure legends

Figure 1. CONSORT diagram. FAS, full analysis set; *FGFR2*, fibroblast growth receptor-2.

Figure 2. Progression-free survival Kaplan-Meier plots (FAS): overall population (A), *FGFR2* polysomy population (B), and *FGFR2* amplification population (C). BID, twice daily; *FGFR2*, fibroblast growth receptor-2.

Figure 3. Overall survival Kaplan-Meier plot (FAS) overall population (A), *FGFR2* polysomy population (B), and *FGFR2* amplification population (C). BID, twice daily; *FGFR2*, fibroblast growth receptor-2.

Figure 4. Analysis of formalin-fixed, paraffin-embedded archival tumor samples from patients with advanced GC in SHINE showing: (A) *FGFR2* expression (log_2 normalized data) of archival tumor sections compared with amplified (SNU16, KATOIII, SUM52) and non-amplified (AGS, SNU-216, SNU-620) cell lines; (B) *in situ* heterogeneity mapping of seven patient samples and an SNU16 GC xenograft section showing tissue classifications and binary heterogeneity maps (non-amplified = blue; amplified = orange) for a large representative field of view for each tumor. The table shows cell count, % amplification (based on ratio ≥2) and average ratio score; and (C) a waterfall plot showing best change in target lesion size for SHINE patients who received AZD4547. *FGFR2*, fibroblast growth receptor-2; FISH, fluorescence *in situ* hybridization; GC, gastric cancer.

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Table 1. Median PFS and OS stratified by *FGFR2* low and high amplification, and polysomy (FAS).

	AZD4547				Paclitaxel			
	Amplification (*n* = 38)			Polysomy (*n* = 20)	Amplification (*n* = 30)			Polysomy (*n* = 15)
	Total (*n* = 18)	Low (*n* = 9)	High (*n* = 9)		Total (*n* = 15)	Low (*n* = 10)	High (*n* = 5)	
PFS								
Median PFS (months)	1.5	1.4	2.0	1.9	2.3	1.9	3.7	3.5
No. events	17	9	8	19	13	10	3	13
Duration of follow-up (months)	1.46	-	-	1.86	1.87	-	-	3.52
OS								
Median OS (months)	4.9	4.9	10.5	6.3	4.6	3.5	NC	7.2
No. deaths	12	6	6	15	9	8	1	9
Duration of follow-up (months)	3.0	2.0	3.4	6.0	3.9	3.5	6.5	6.6

FAS, full analysis set; *FGFR2*, fibroblast growth factor receptor-2; NC, non-calculable; OS, overall survival; PFS, progression-free survival.

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Table 2. Best objective response stratified by *FGFR2* low and high amplification, and polysomy (FAS[a]).

		AZD4547			Paclitaxel		
		Low amplification (*n* = 9)	**High amplification (*n* = 9)**	**Polysomy (*n* = 20)**	**Low amplification (*n* = 10)**	**High amplification (*n* = 5)**	**Polysomy (*n* = 15)**
Response	Complete response, *n* (%)	0	0	0	0	0	0
	Partial response, *n* (%)	0	0	1 (5.0)	1 (10.0)	2 (40.0)	4 (26.7)
Non-response	Stable disease ≥8 weeks, *n* (%)	1 (11.1)	2 (22.2)	5 (25.0)	3 (30.0)	2 (40.0)	5 (33.3)
	Progression, *n* (%)	8 (88.9)	6 (66.7)	14 (70.0)	6 (60.0)	1 (20.0)	4 (26.7)
	RECIST progression	6 (66.7)	5 (55.6)	13 (65.0)	2 (20.0)	1 (20.0)	4 (26.7)
	Death	2 (22.2)	1 (11.1)	1 (5.0)	4 (40.0)	0	0
	Not evaluable, *n* (%)	0	1 (11.1)	0	0	0	2 (13.3)

[a]FISH re-scoring (removal of the cluster rule) to detect *FGFR2* amplification resulted in the identification of three patients in the FAS who no longer met the criteria for polysomy or amplification.

FAS, full analysis set; *FGFR2*, fibroblast growth factor receptor-2; FISH, fluorescence *in situ* hybridization; RECIST, Response Evaluation Criteria In Solid Tumors.

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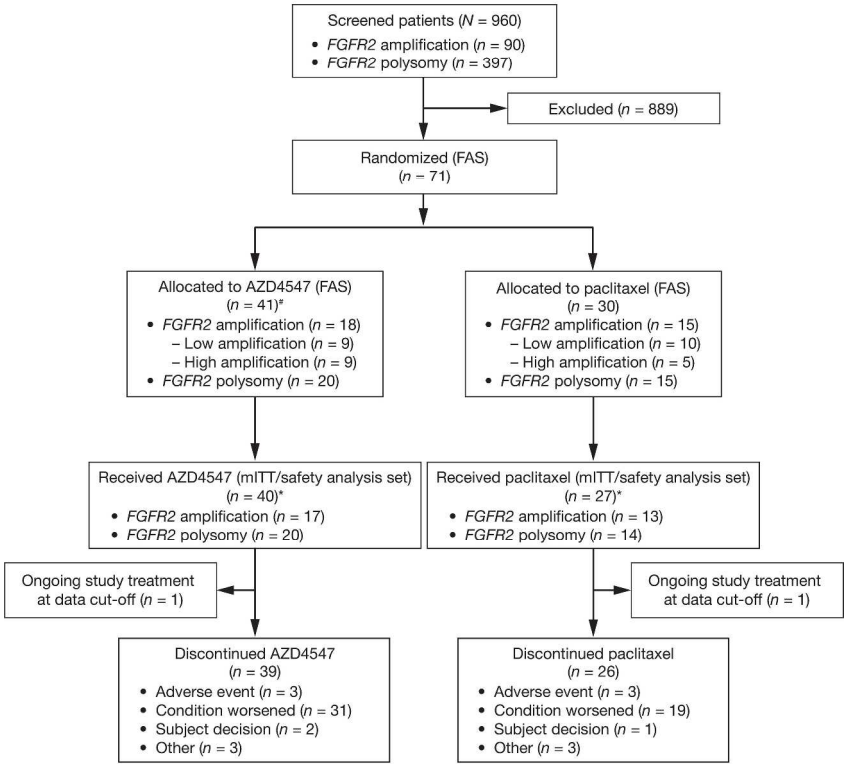
Figure 1



*Three patients did not receive study therapy because they died prior to administration (one in the AZD4547 arm and two in the paclitaxel arm); One patient in the paclitaxel arm had 'Other' recorded with no further details;
#Including three patients who no longer met the criteria for polysomy or amplification.

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Figure 2

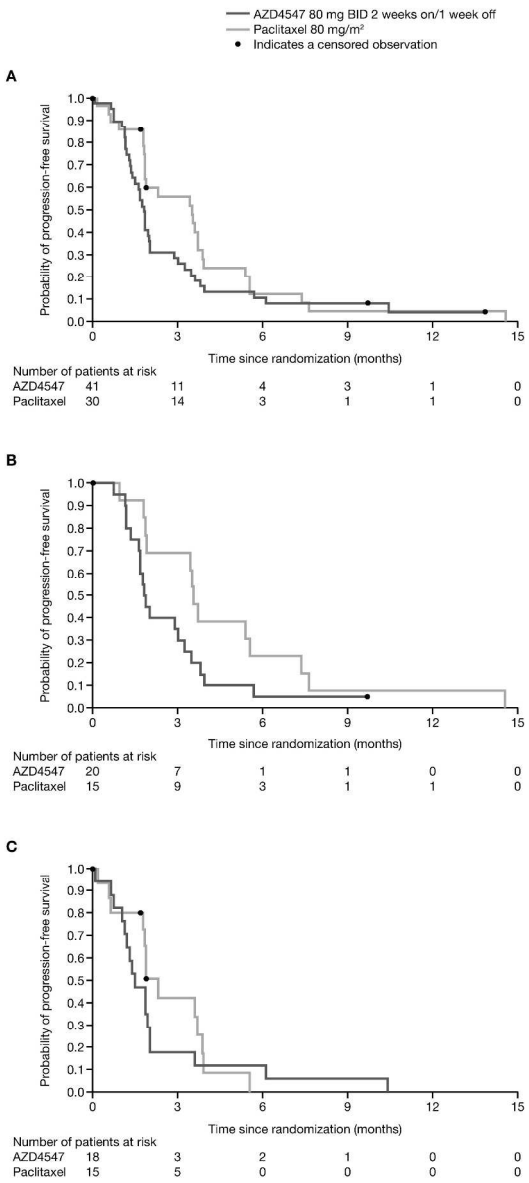

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Figure 3

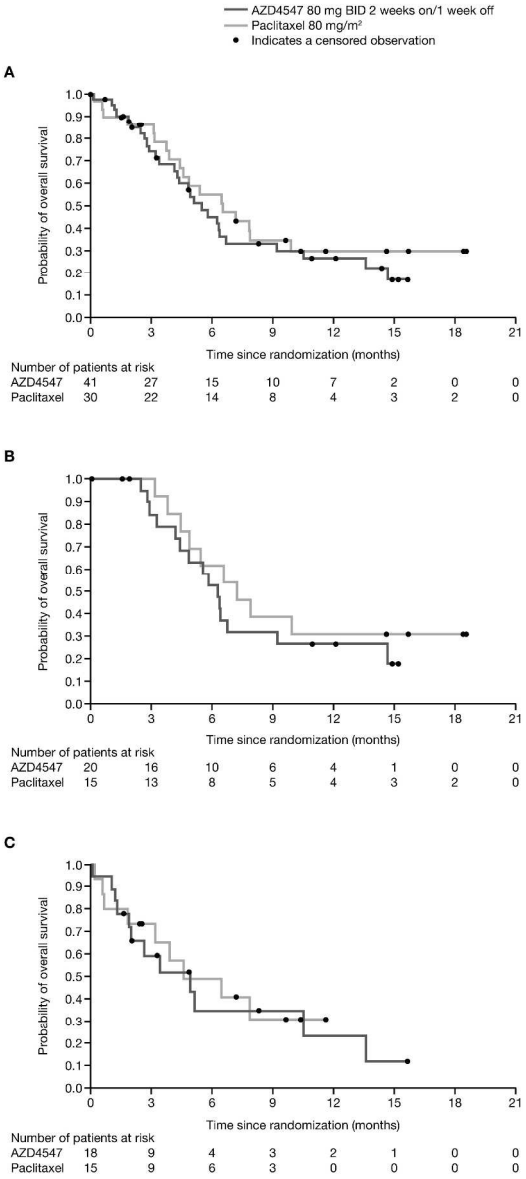

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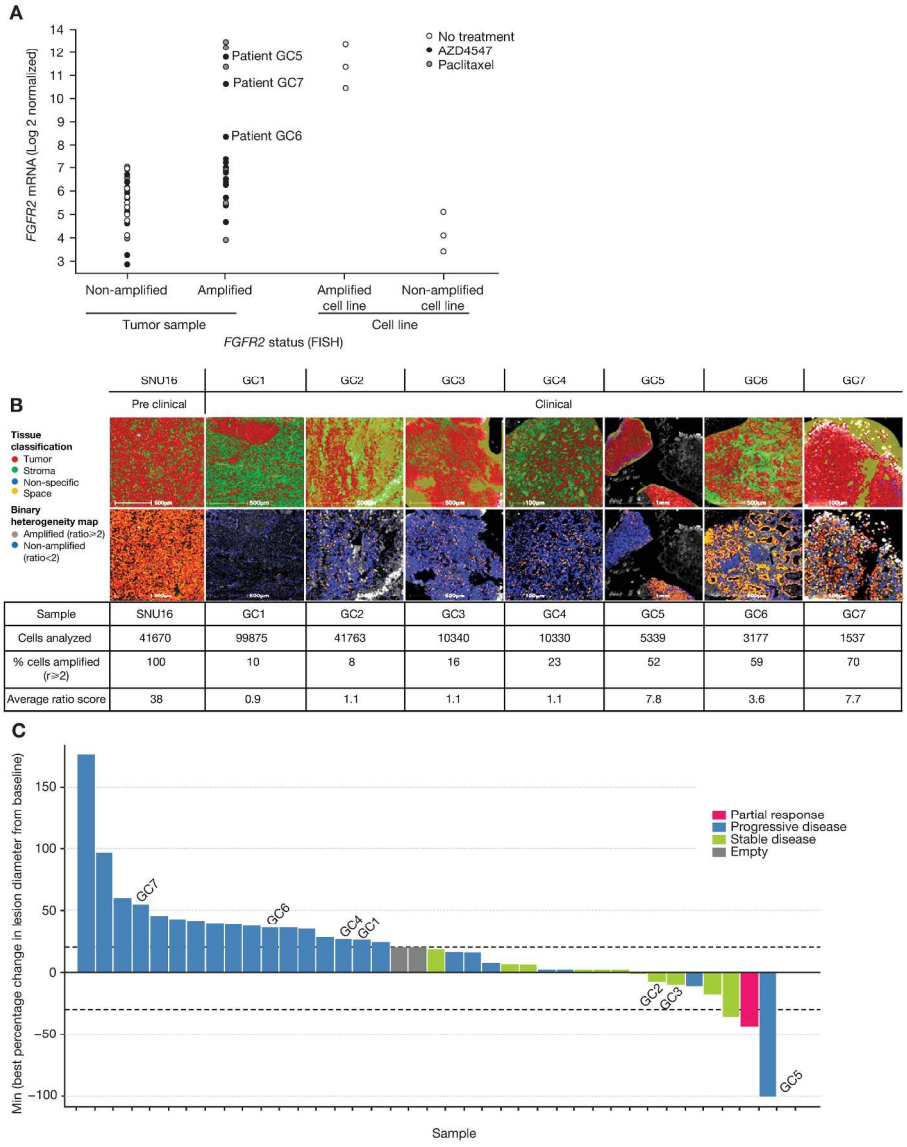
Figure 4



Sample	SNU16	GC1	GC2	GC3	GC4	GC5	GC6	GC7
Cells analyzed	41670	99875	41763	10340	10330	5339	3177	1537
% cells amplified (r>2)	100	10	8	16	23	52	59	70
Average ratio score	38	0.9	1.1	1.1	1.1	7.8	3.6	7.7

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Supplementary Material

Materials and Methods

Fluorescence *in situ* hybridization (FISH) scoring system

Tumor fibroblast growth factor receptor (FGFR) status was determined by centralized FISH screening with a scoring system similar to that used for epidermal growth factor receptor and human epidermal growth factor receptor 2 (*HER2*) [1]. Tumor sections were scanned at low magnification to identify areas of gene copy number gain and then 50 cell nuclei were counted.

Due to the difficulties in consistently applying the scoring system for cluster definition the FISH scoring system was reviewed during the study and the cluster definition removed from the amplification category, hence the definition for *FGFR2* amplification was refined to include only: *FGFR2*/CEN10 ratio ≥2. Tumor samples from all randomized patients were re-scored and patients who no longer met the criteria for amplification were excluded from the final analysis.

Patients in the *FGFR2* amplification cohort were randomized 3:2 to AZD4547 or paclitaxel, within the *FGFR2* low- and high-level amplification strata. Patients in the *FGFR2* polysomy cohort were randomized 1:1 to AZD4547 or paclitaxel.

Results

Participants

Four patients were randomized but did not receive randomized treatment and therefore the modified intention-to-treat (mITT) and safety analysis population consisted of 67 patients (AZD4547 $n = 40$ [59.7%], polysomy $n = 20$, amplification $n = 17$; paclitaxel $n = 27$ [40.3%], polysomy $n = 14$, amplification $n = 13$). Patients randomized to the two treatment groups were generally well balanced with respect to demographic characteristics (supplementary Table S1).

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Safety

AEs and serious AEs related to study treatment occurred at similar rates in both treatment arms (supplementary Table S2). Six (15%) patients in the AZD4547 arm experienced retinal pigmented epithelium detachment (RPED), with the majority of cases of Common Terminology Criteria for Adverse Events (CTCAE) Grade 1/2. No patients in the paclitaxel arm developed the condition. AEs related to study treatment that led to treatment discontinuation occurred in 2 patients in each arm (5.0% for AZD4547 and 7.4% for paclitaxel). Two patients in the AZD4547 arm and one patient in the paclitaxel arm experienced an AE (intestinal hemorrhage, arterial disorder, or asthenia) with an outcome of death. None of the deaths were considered by the investigator to be causally related to the study drug.

Hematology and clinical chemistry

The greatest incidences of changes classified as CTCAE-Grade 3/4 were reported for leukocytes decreased (2 [5.0%] for AZD4547; 4 [15.4%] for paclitaxel), neutrophils decreased (4 [10.3%] for AZD4547; 4 [17.4%] for paclitaxel), lymphocytes decreased (6 [15.4%] for AZD4547; 3 [13.0%] for paclitaxel), alkaline phosphatase increased (5 [13.2%] for AZD4547; 2 [7.7%] for paclitaxel), and phosphate increased (4 [10.0%] for AZD4547; 1 [4.0%] for paclitaxel).

Dose modification occurred more frequently in the AZD4547 arm (13 [32.5%] patients) compared with the paclitaxel arm (6 [22.2%] patients). Eleven (27.5%) patients in the AZD4547 arm and 5 (18.5%) patients in the paclitaxel arm had their study dose interrupted. Five (12.5%) patients in the AZD4547 arm and 3 (11.1%) patients in the paclitaxel arm had dose reduction. The occurrence of an adverse event (AE) was the most common reason for dose modifications, dose reductions, and dose interruptions.

Pharmacokinetic analysis

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A clear increase in plasma phosphate levels was observed during cycles 1, 2, and 3 of AZD4547 administration with a return to normal levels during the week off while no corresponding increase was observed with paclitaxel treatment (supplementary Figure S1). No significant changes from baseline were observed in plasma bFGF and FGF23 in either the AZD4547 or paclitaxel arm (data not shown). There was no apparent difference in AZD4547 exposure with respect to surgery versus no surgery and surgery type. PK data displayed high variability due, in part, to dose reductions in some patients from 80 mg to 40 mg twice daily (BID).

Exploratory survival analysis

In agreement with previous reports [2–4], follow-up of non-randomized patients who underwent FISH pre-screening in the SHINE study showed a trend for *FGFR2* amplification to be inversely correlated with overall survival. However this was not statistically significant by multivariate analysis (aggregated HR non-amplified versus amplified: 1.15 [0.81–1.63]; $p = 0.437$) (supplementary Figure S2).

References

1. Varella-Garcia M. Stratification of non-small cell lung cancer patients for therapy with epidermal growth factor receptor inhibitors: the EGFR fluorescence in situ hybridization assay. Diagn Pathol. 2006;1:19.

2. Su X, Zhan P, Gavine PR, Morgan S, Womack C, Ni X, et al. FGFR2 amplification has prognostic significance in gastric cancer: results from a large international multicentre study. Br J Cancer 2014;110:967–75.

3. Jung EJ, Jung EJ, Min SY, Kim MA, Kim WH. Fibroblast growth factor receptor 2 gene amplification status and its clinicopathologic significance in gastric carcinoma. Hum Pathol 2012;43:1559–66.

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4. Matsumoto K, Arao T, Hamaguchi T, Shimada Y, Kato K, Oda I, et al. FGFR2 gene amplification and clinicopathological features in gastric cancer. Br J Cancer 2012;106:727–32.

Supplementary Figure legend

Figure S1. Modulation of absolute plasma phosphate levels in the AZD4547 treatment arm during on- and off-drug periods (A) compared with the paclitaxel treatment arm (B).

Figure S2. Overall probability of survival Kaplan-Meier plot by *FGFR2* amplification and gene copy number analyzed by FISH; all pre-screened patients who were not randomized. Aggregated hazard ratio non-amplified versus amplified: 1.15 [0.81–1.63]; $p = 0.437$; multivariate analysis. *FGFR2*, fibroblast growth factor receptor-2; FISH, fluorescence *in situ* hybridization.

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Table S1. Clinical characteristics and baseline demographics (FAS).

	AZD4547 (*n* = 41)	**Paclitaxel (*n* = 30)**	**Total (*n* = 71)**
Male, *n* (%)	29 (70.7)	22 (73.3)	51 (71.8)
Mean (SD) age, years	60.6 (11.4)	61.9 (10.7)	61.2 (11.0)
Prior chemotherapy[a]			
Capecitabine	23 (56.1)	15 (50.0)	38 (53.5)
Cisplatin	21 (51.2)	18 (60.0)	39 (54.9)
Fluorouracil	15 (36.6)	9 (30.0)	24 (33.8)
Oxaliplatin	15 (36.6)	7 (23.3)	22 (31.0)
Epirubicin	11 (26.8)	9 (30.0)	20 (28.2)
Number of prior chemotherapy regimens			
1	34 (82.9)	24 (80.0)	58 (81.7)
2	5 (12.2)	2 (6.7)	7 (9.9)
3	0	1 (3.3)	1 (1.4)
Prior surgical procedures			
Gastrectomy	15 (36.6)	8 (26.7)	23 (32.4)
Overall disease classification			
Metastatic[a]	40 (97.6)	30 (100)	70 (98.6)
Respiratory	10 (24.4)	5 (16.7)	15 (21.1)
Hepatic[b]	25 (61.0)	15 (50.0)	40 (56.3)
Lymph nodes	21 (51.2)	18 (60.0)	39 (54.9)
Peritoneum	8 (19.5)	10 (33.3)	18 (25.4)
Locally advanced	1 (2.4)	0	1 (1.4)

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[a]Reported in ≥10 patients; [b]Including gall bladder.

Other lung/liver classifications not included within the 'respiratory' or 'hepatic' disease classifications: lung (*n* = 1), lung and liver metastases (*n* = 1), liver (*n* = 1), lung and pleura metastases (n=1), lung, liver, mediastinum (n=1).

FAS, full analysis set; SD, standard deviation.

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Table S2. AEs reported in ≥10% of patients in either treatment arm (safety analysis; *n* = 67).

	AZD4547 (*n* = 40)	**Paclitaxel (*n* = 27)**
Any AE causally related to study treatment, *n* (%)	29 (72.5)	19 (70.4)
Any AE of CTCAE Grade ≥3 causally related to study treatment, *n* (%)	7 (17.5)	5 (18.5)
Any SAE causally related to study treatment, *n* (%)	1 (2.5)	1 (3.7)
Decreased appetite, *n* (%)	16 (40.0)	8 (29.6)
Asthenia, *n* (%)	11 (27.5)	5 (18.5)
Nausea, *n* (%)	10 (25.0)	6 (22.2)
Constipation, *n* (%)	10 (25.0)	5 (18.5)
Stomatitis, *n* (%)	10 (25.0)	2 (7.4)
Abdominal pain, *n* (%)	9 (22.5)	5 (18.5)
Upper abdominal pain, *n* (%)	9 (22.5)	0
Dry mouth, *n* (%)	9 (22.5)	0
Vomiting, *n* (%)	8 (20.0)	5 (18.5)
Anemia, *n* (%)	7 (17.5)	6 (22.2)

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Increased aspartate aminotransferase, *n* (%)	7 (17.5)	0
Fatigue, *n* (%)	6 (15.0)	8 (29.6)
Diarrhea, *n* (%)	6 (15.0)	6 (22.2)
Dysgeusia, *n* (%)	6 (15.0)	4 (14.8)
Retinal pigment epithelium detachment, *n* (%)	6 (15.0)	0
Increased alanine aminotransferase, *n* (%)	6 (15.0)	0
Peripheral edema, *n* (%)	4 (10.0)	2 (7.4)
Pyrexia, *n* (%)	4 (10.0)	2 (7.4)
Dyspepsia, *n* (%)	4 (10.0)	1 (3.7)
Headache, *n* (%)	4 (10.0)	1 (3.7)
Increased blood alkaline phosphatase, *n* (%)	4 (10.0)	1 (3.7)
Dry eye, *n* (%)	4 (10.0)	0
Alopecia, *n* (%)	2 (5.0)	13 (48.1)
Neutropenia, *n* (%)	2 (5.0)	9 (33.3)
Insomnia, *n* (%)	2 (5.0)	3 (11.1)

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Back pain, *n* (%)	1 (2.5)	6 (22.2)
Peripheral neuropathy, *n* (%)	1 (2.5)	4 (14.8)
Lower respiratory tract infection, *n* (%)	0	3 (11.1)
Myalgia, *n* (%)	0	3 (11.1)
Peripheral sensory neuropathy, *n* (%)	0	3 (11.1)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; SAE, serious adverse event.

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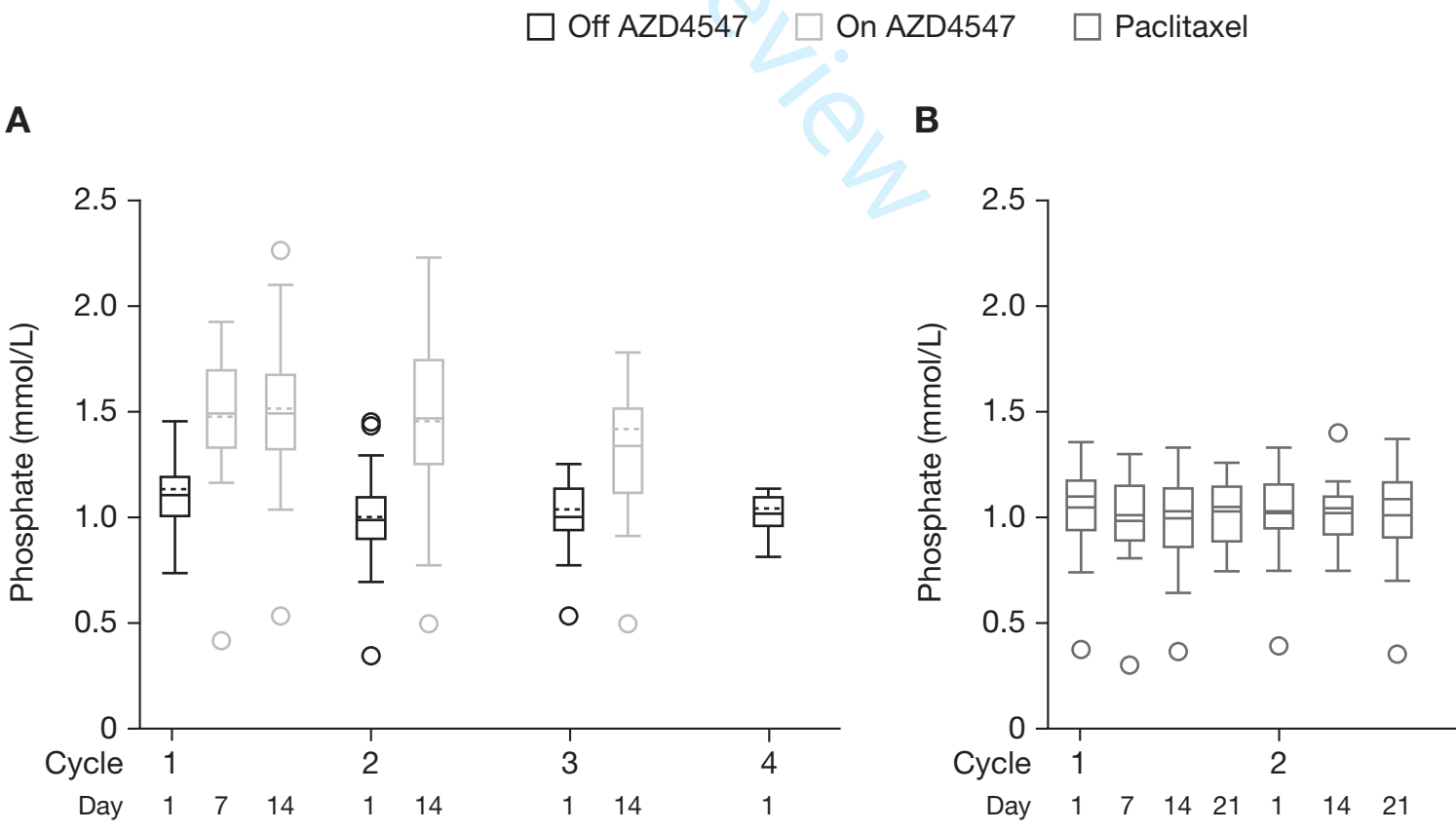
Supplementary Figure 1



Horizontal line: median; Box: Q1–Q3

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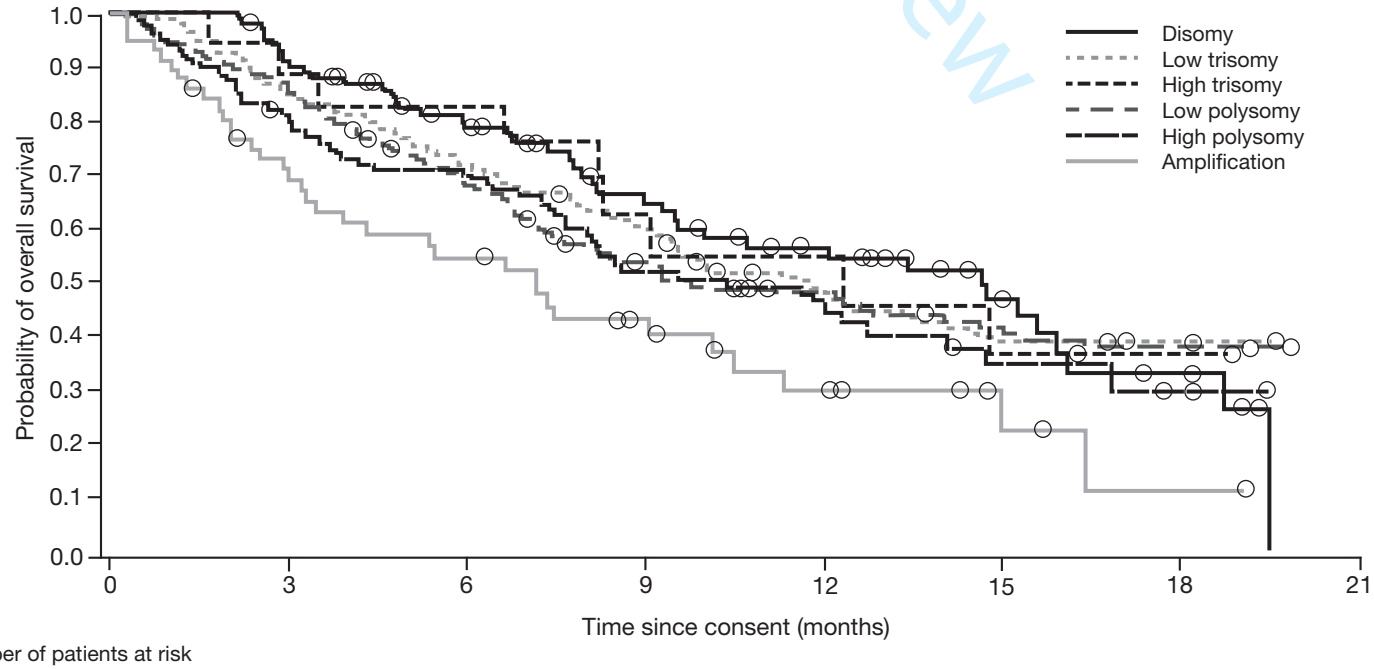
Supplementary Figure 2



Number of patients at risk

Disomy	111	90	63	40	30	15	6	0
Low trisomy	198	145	102	70	46	34	10	0
High trisomy	18	15	13	8	6	3	1	0
Low polysomy	253	175	122	75	48	35	12	0
High polysomy	110	79	61	38	21	11	5	0
Amplification	57	36	25	16	8	3	1	0

○ Indicates a censored observation

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